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Onderwerp
Assessment of an EFSA opinion on the cultivation of Bt11 maize

Geachte heer Van Geel,

Naar aanleiding van de adviesvraag van het ministerie van VROM betreffende de beoordeling van het EFSA advies over de teelt van BT11 maïs (C/F/96/05.10), deelt de COGEM u het volgende mee.

Samenvatting:
De COGEM is gevraagd te adviseren betreffende een advies van de European Food Safety Authority (EFSA) over teelt, veevoedergebruik en verwerking van de genetische gemodificeerde maïslijn Bt11. De Europese Commissie heeft de EFSA om een oordeel gevraagd omdat verschillende lidstaten, waaronder Nederland, vragen hadden gesteld na beoordeling van deze maïslijn. Mede op basis van dit advies zal de Nederlandse positie bij stemming van de lidstaten van de Europese Unie worden bepaald. De Bt11 maïslijn is door de inbouw en de expressie van het *cry1Ab* gen minder gevoelig voor plaaginsecten, met name de Europese maïsboorder. Daarnaast is Bt11 resistent tegen het herbicide glufosinaat ammonium als gevolg van expressie van het bacteriële *pat* gen.

Maïs kent geen wilde verwanten in Nederland en opslag van maïsplanten is hier niet van landbouwkundige betekenis. Verwildering van maïs in Nederland is nog nooit waargenomen. Er zijn geen redenen om aan te nemen dat de modificatie het verwilderingspotentieel vergroot. De ingebrachte genen en de genproducten kennen een geschiedenis van veilig gebruik. De vragen die Nederland eerder had gesteld over de effecten op niet-doelorganismen en persistentie van het Cry1Ab eiwit in de bodem bij beoordeling van het desbetreffende marktdossier, zijn in het EFSA advies afdoende beantwoord. De COGEM acht derhalve de risico's voor mens en milieu bij de teelt, veevoedergebruik en verwerking van onderhavige maïslijn verwaarloosbaar klein.

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De door de COGEM gehanteerde overwegingen en het hieruit voortvloeiende advies treft u hierbij aan als bijlage.

Hoogachtend,

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Title: Assessment of an EFSA opinion on the cultivation of Bt11 maize

COGEM advice: CGM/050816-01

The Netherlands Commission on Genetic Modification (COGEM) is asked by the Dutch Competent Authority to issue advice on an opinion of the European Food Safety Authority (EFSA) concerning the cultivation, feed and industrial processing of the genetically modified maize line Bt11. An assessment of Bt11 maize was requested by the European Commission because of questions raised by several Member States (including The Netherlands) following the evaluations at the national level.

Bt11 maize contains genes (cry1Ab and pat) conferring resistance to certain Lepidopteran insects, and tolerance to herbicides containing the active ingredient glufosinate.

In The Netherlands, no wild relatives of maize are present and establishment of maize plants in the wild has never been observed. There are no reasons to assume that the inserted traits will increase the potential of the maize line to run wild. The former objections of The Netherlands on the effects on non target organisms and the persistence in the soil of the Cry1Ab protein are sufficiently answered in the EFSA opinion

Considering the above-mentioned, COGEM is of the opinion that the proposed cultivation, feed and industrial processing of the maize line Bt11 does not pose a significant risk for human health and the environment.

Introduction

The opinion of the European Food Safety Authority (EFSA) concerns the cultivation, feed and industrial processing of the genetically modified maize line Bt11. An assessment of Bt11 maize was requested by the European Commission because of questions raised by several Member States (including the Netherlands) following the evaluations at the national level.

Bt11 maize contains a *cry1Ab* gene conferring resistance to specific Lepidopteran pests, particularly the European corn borer (*Ostrinia nubilalis*) and *Sesamia* spp. Furthermore, Bt11 maize contains the *pat* gene conferring tolerance to herbicides containing the active ingredient glufosinate ammonium.

Previous COGEM advices

In the past COGEM has advised positively concerning the genetically modified Bt11 maize import and processing (CGM/970204-06). However, COGEM has negatively advised on a notification for the cultivation of Bt11 maize (CGM/990707-05, CGM/030822-01, CGM/040212-01). The reason for these negative advices was the lack of information in the notification of possible effects of the Cry1Ab protein on non-target organisms under field conditions. Moreover, information was lacking on the persistence of the Cry1Ab protein in soil.

Aspects of the crop

Maize (*Zea mays* L.) is a member of the grass family *Poaceae* and cultivation of maize, as an agricultural crop, originated in Central America. Maize is predominantly wind pollinated, although, insect pollination can not be completely excluded (1; 2). According to literature, pollen viability varies between 30 minutes and 9 days (2-4). There are no wild relatives of maize in Europe and, therefore, it is not possible that maize will hybridise with other species.

The appearance of volunteers is very rare under Dutch conditions. Grains do not possess dormancy, resulting in a short persistence. Furthermore, after harvesting of fodder maize only few seeds remain on the field (1). In the Netherlands, maize has never established itself in the wild.

Molecular characterisation

Origin and function of the introduced genes

Bt11 maize was generated by transformation of *Zea mays* protoplasts according to the protocol described by Negrutiu et al., 1987 (5). For this purpose a pZO1502 vector, derived from pUC18, was used. The pZO1502 vector contains, besides the pUC18 sequences, the following elements:

- the *pat* gene, an artificial gene, coding for phosphinotricine acetyltransferase, originating from *Streptomyces viridochromogenes*. It is regulated by a constitutive 35S promotor of the C*auliflower mosaic virus* (CaMV) and the terminator of the *nopaline synthase* gene derived from *Agrobacterium tumefaciens*.
- *cry1Ab* gene, a modified version of the gene originating from *Bacillus thuringiensis* subsp. *kurstaki* stock HD-1, coding for the Bt toxin Cry1Ab. The gene is regulated by a constitutive 35S promotor of the CaMV and the terminator of the *nopaline synthase* gene derived from *A. tumefaciens*.

Properties of the introduced genes conferring insect resistance

Bt11 was genetically modified with the *cry1Ab* gene derived from *B. thuringiensis*. The produced Cry1Ab, a δ-endotoxin, is lethal to insects of the *Lepidoptera* order, including the European corn borer (*O. nubilalis*) and the Mediterranean corn borer (*Sesamia nonagriodes*). The δ-endotoxin selectively binds to receptors located in the midgut of susceptible insects. Following binding, the gut is perforated causing death of the insect within 48 to 72 hours (6).

The European corn borer (ECB) and the Mediterranean corn borer (MCB) are the major pests of maize in many maize-growing regions of Central Europe and countries around the Mediterranean Basin (7; 8). Damage to the maize plant is mainly caused by feeding of the ECB or MCB larvae in the stalk or ear shank. Yield losses are largely attributable to a reduction in kernel number and weight owing mainly to physiological disruption of the plant growth and only to a minor extent to broken stalks, dropped ears and larval feeding on the grain (9; 10).

Properties of the introduced genes conferring herbicide tolerance

Bt11 maize was genetically modified with the *pat* gene encoding the PAT protein (phosphinothricin-N-acetyl transferase). In this way tolerance was obtained to glufosinate ammonium herbicides.

Glufosinate ammonium is a broad-spectrum herbicide and is used to control a wide range of weeds after the crop emerges or for total vegetation control on land not used for cultivation. It inhibits the activity of the enzyme, glutamine synthetase, which is necessary for the production of glutamine and for ammonia detoxification. The application of glufosinate leads to reduced glutamine and increased ammonia levels in the plant tissues. This causes photosynthesis to stop and the plant dies within a few days (11). The PAT protein acetylates L-phosphinothricin, the active isomer of the glufosinate ammonium herbicide, resulting in tolerance of transgenic plants to the herbicide (12).

Molecular analysis

The molecular analysis was already evaluated in previous COGEM reports and COGEM agreed with the outcome of this analysis. COGEM concluded that the application of Bt11 maize for cultivation, feed and industrial processing, does not pose a significant risk for human health and the environment.

Advice

The present opinion of the European Food Safety Authority (EFSA) concerns the cultivation, feed and industrial processing of the genetically modified maize line Bt11. There are no reasons to assume that the inserted traits will increase the potential of the maize line to establish feral populations. The inserted genes and their gene products possess a history of safe use and it is sufficiently proven that no toxic or allergenic products are formed. The EFSA opinion provides an extensive and welcome overview of the effects of Bt toxins. Scientific studies dealing with predator and parasitoids abundances in Bt corn fields show no negative effect on non-target organisms. Furthermore, data are presented that prove that accumulation of Bt toxin in the soil does not negatively affect other soil organisms. In the opinion of COGEM this overview provides sufficient evidence to dismiss its former objections towards the lack of information of the Cry1Ab protein on non-target organisms under field conditions and the lack of information on the persistence of the Cry1Ab protein in the soil. In view of the above-mentioned, COGEM is of the opinion that the risks for the environment and human health associated with the cultivation, feed and industrial processing of Bt11 maize are negligible.

Additional remarks

Although COGEM has a positive opinion on the EFSA opinion and dismisses the former objections, there are some points in the EFSA opinion which, to the opinion of the experts of COGEM, do not reflect the scientific literature completely. These points do not affect the thrust of the argument of the current EFSA opinion. However, they might be of interest, in view of possible future notifications. These points of concern regard the paragraph 5.2.4 'Interactions of the GM plant with non-target organisms' and the paragraph 5.2.5 'Potential interaction with the abiotic environment and potential effects on biogeochemical processes'.

Paragraph 5.2.4.a concerns general information about Bt or Cry toxins and does not concern Cry1Ab in particular. However, it is known that Cry toxins have different effects. In the first paragraph of 5.2.4.b, the effect of pollen dose is not mentioned. Yet, especially dose-response studies are important to assess the degree of effect of the toxin. In paragraph 5.2.4.b it is stated that 'No evidence of accumulation of Bt toxins in the food chain has been reported and it is not expected as the toxin is an easily degradable protein'. The study by Dutton et. al. 2002 (13) has shown this to be untrue. Spider mites that feed on Bt11 maize accumulate large doses of Bt toxin (Cry1Ab) and by feeding on these toxin-accumulated spider mites, lacewings were exposed to high doses of the toxin. The lacewings were not negatively affected, but the conclusion in the EFSA opinion that there is no accumulation of Bt toxins in the food chain has been documented to be incorrect. More recently, accumulation of Cry1ab in the food chain has been documented for a different food chain as well (14).

In paragraph 5.2.5 the EFSA report states that 'Cry proteins are rapidly decomposed in the soil'. Although the study by Glare and O'Callaghan, 2000 (15) supports this, the study by Zwahlen et al., 2003 (16) that is cited in the next sentence and the study by Saxena and Stotzky, 2001 (cited in the EFSA opinion) (17) show that accumulation of Bt toxin in the soil does occur. Although, the accumulated toxin in the soil did not negatively affect earthworms and nematodes, the accumulation did occur which contradicts the statement in the previous sentence. In fact, it has been reported earlier that the soil type affects Bt toxin accumulation in the soil (18). Tapp and Stotzky (1998) reported biological activity of Bt toxins in the soil in terms of insect mortality up to 234 days. Therefore the general comment on rapid decomposition does not reflect our knowledge completely and ignores examples for soil types where accumulation does occur.

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